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Temperature-induced gametogenesis in the Yezo scallop

by G.I. Viktorovskaya and V.V. Yevdokimov (TINRO**)

The reproduction cycle of the Yezo scallop, Patinopecten jessoensis, which is regareded as the period between two consecutive spawnings, is equal to one year in the Peter the Great Gulf (Dzyuba, 1971). During this period, the growth rates of the sex cells of the bivalves vary significantly, mainly under the influence of the habitat environment, where the temperature factor plays a decisive role.

The purpose of our experiment was to establish the dependence of the reproductive process (and the physiological activity of the species as a whole) on the temperature of its environment when sex cells capable of maturing are present in the gonad. The experiments were carried out to obtain (much sooner than under natural conditions) mature sex cells in scallops that have already spawned, and to confirm their viability.

Material and Method

The experiment was carried out in aquariums with running water, in which the temperature was controlled automatically;

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the water volume was maintained at not less than 2 litres per scallop. The mollusks were fed a mixture of algae. Two to threee-year-old scallops were caught in Alekseyev Bay in the Peter the Great Gulf at a depth of 5-10 m. We performed three experiments lasting 21-66 days (in autumn, winter and spring). Ninety-five specimens of the Yezo scallop were used in the experiments.

Gametogenesis was induced by increasing the water temperature to 10-12°C, i.e. to the conditions of natural spawning (Viktorovskaya et al., 1982). The effect of light on the reproductive activity of the animals was not taken into consideration; the periods of daylight and darkness characteristic of Vladivostok were maintained throughout all the experiments.

Five specimens of each sex were dissected prior to the experiment. The turgor, colour and mass of the gonads were determined, and then fragments were cut from the latter for microscopic analysis. Depending on the length of the experiment, auxiliary observations were carried out at different times in each case, and after 10, 21 and 30 days in all of the experiments. (4)

During the experiment, we measured the sizes of the acini in the females and males, and also examined the sizes of the zones of reproduction, growth and formation in the males. In the female acini, we counted the total number of sex cells, and measured the sizes of the cells and their nuclei.

The results were subjected to statistical analysis. An embryologic experiment was carried out to determine the viability of the sex cells obtained in the course of the experiments.

This was achieved by clearing the genital products in the animals at the end of the experiments by Yamamoto's method (1966), and then artificially fertilizing the gametes and studying the early stage of ontogenesis up to spatfall. The development of the embryos took place in containers with filtered water with a density of one embryo per ml of water at 10°C. One-third of the water in the containers was replaced with fresh water every day. The larvae were supplied with food upon transition to exogenous feeding.

The percentage of abnormalities was estimated according to the stages of development.

Results and Discussion

We found that the mass of the gonad increased consistently during the experiment (table 1). The development of the gonad, i.e. the increase in its mass per unit of time, takes place more rapidly under artificial conditions as compared with a natural environment.

The size of the acini increases simultaneously with the mass of the gonad both in the females and males (table 1), which is also an indication of gonadal development. The size of the acini during the experiments increased significantly ($P < 0.001$) in comparison with their initial size.

To determine the qualitative changes in the female gonad, we divided all the sex cells into parietal and free-lying oocytes. The parietal oocytes predominated in the acini at the beginning of each experiment, i.e. in scallops with an immature gonad (Fig. 1, a, A). As the water temperature increased, the number

of these cells decreased, and dropped sharply at the end of each experiment.

The free-lying oocytes are cells characterized by terminating trophoplasmic growth. Some of them undergo resorption with the formation of a trophic substrate, i.e. the same occurs as in the process of gametogenesis in a natural environment. The number of free-lying oocytes increased noticeably at the intermediate stages, and decreased by the end of the experiment. By the end of the experiment, almost the entire acinus was filled with free-lying oocytes (Fig. 1, a, B).

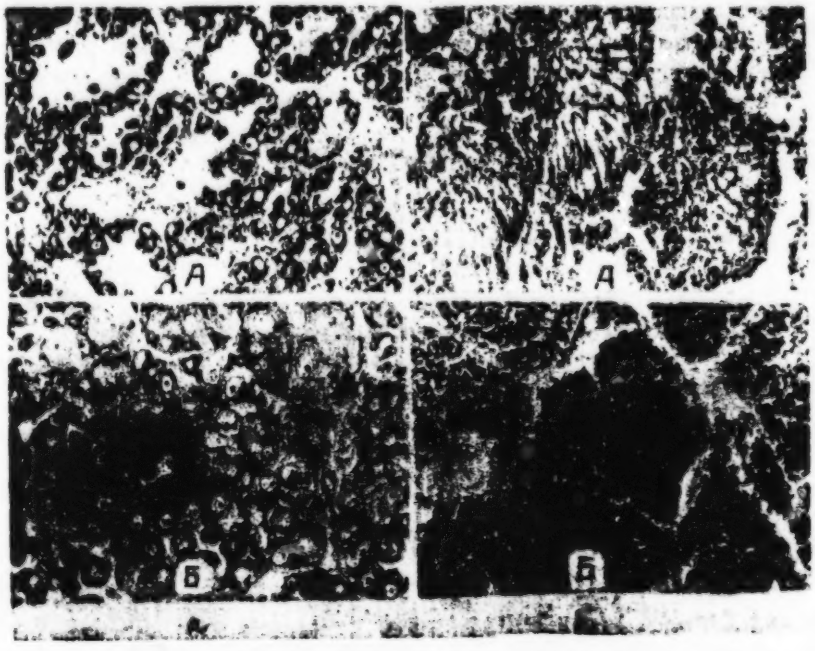

Fig. 1. Microphotograph of gonads in Patinopecten jessoensis:
a - females before the experiment (A - parietal and free-lying oocytes can be seen) and at the end of the experiment (B - mature sex cells predominate); b - males before the experiment (A - a small reproduction zone can be seen) and at the end of the experiment (B - the greater part of a follicule is occupied by mature spermatozoa). Ehrlich hematoxylin staining, oc. x10, obj. x10.

Table 1. Morphometric indices of gonadal development in *P. jessoensis* during the experiment

Indices		Experiment 1		Experiment 2			Experiment 3		Control (spawning period)
		5.11.81	5.12.81	11.1.82	18.2.82	28.3.82	7.4.82	28.4.82	
Female gonads									
Mass of gonad (g)	M	7,1	8,4	9,6	7,4	10,5	7,9	10,4	10,5
	m	0,3	0,5	0,4	0,2	0,3	0,4	0,3	0,5
	p	-	<0,001	<0,001	-	<0,001	-	<0,001	-
Volume of acini (mm^3)	M	0,18	0,27	0,29	0,27	0,31	0,28	0,31	0,31
	m	0,01	0,002	0,002	0,02	0,003	0,01	0,03	0,03
	p	-	<0,001	>0,5	-	<0,001	-	>0,5	-
cell (μm^3)	M	29514	49280	102431	40590	114474	82833	114478	114302
	m	1110	2367	2905	1116	2544	1643	2074	2236
	p	-	<0,001	<0,001	-	<0,001	-	<0,001	-
nucleus (μm^3)	M	5391	6831	8511	6590	8942	8367	8749	8702
	m	294	245	194	388	260	213	210	196
	p	-	<0,001	<0,001	-	<0,001	-	<0,001	-
Nucleus/plasma ratio		0,19	0,15	0,08	0,17	0,07	0,1	0,07	0,07
Male gonads									
Mass of gonad (g)	M	6,9	7,9	9,3	7,3	10,4	7,9	10,5	10,4
	m	0,2	0,4	0,5	0,3	0,5	0,3	0,4	0,5
	p	-	>0,5	<0,001	-	<0,001	-	<0,001	-
Volume of acinus (mm^3)	M	0,17	0,25	0,28	0,25	0,30	0,27	0,30	0,30
	m	0,01	0,001	0,002	0,02	0,003	0,001	0,003	0,003
	p	-	<0,001	>0,5	-	<0,001	-	<0,001	-
zone of reproduction and growth (mm^3)	M	0,07	0,23	0,07	0,24	0,05	0,23	0,04	0,05
	m	0,01	0,005	0,004	0,001	0,005	0,003	0,01	0,01
	p	-	<0,001	<0,001	-	<0,001	-	<0,001	-
zone of formation (mm^3)	M	-	-	0,21	-	0,25	0,04	0,26	0,26
	m	-	-	0,003	-	0,005	0,01	0,005	0,003
								<0,001	
zone free of sex cells (mm^3)	M	0,10	0,02	-	0,01	-	-	-	-
	m	0,01	0,001	-	0,002	-	-	-	-
Optical density of cells in zone of formation	M	-	-	0,92	-	1,08	2,3	1,07	1,07
	m	-	-	0,003	-	0,01	0,02	0,001	0,01
								<0,001	
Coefficient of maturity		-	-	0,19	-	0,27	0,09	0,28	0,28

(7)

The consistent increase in the mean volumes of the cells and their nuclei should be regarded as an obvious indicator of progressive gonadal development (see table 1).

During spermatogenesis, differentiation proceeds from the periphery towards the centre of the acinus. Spermatogonia are distributed near the basal membrane, forming a zone of reproduction, beyond which lies the growth zone represented by primary and secondary spermatocytes. In the centre of the acini, the spermatids of a spermatozoon make up the zone of formation.

Spermatogenesis is activated as a result of heat stimulation. The zones of growth and formation expand noticeably, and we observe an increase in gonadal maturity in all the experiments from beginning to end. The acini gradually fill up with (8) spermatozoa, and the number of spermatocytes and spermatogonia decreases (see table 1 and fig. 1,b).

Consequently, the increase in the mass of the gonad and the size of the acini, as well as the progressive growth followed by maturation of the cells all point to the stimulating effect of temperature on gonadal development.

The viability of the sex cells that mature in an artificial environment has been confirmed by embryologic observations (fig. 2, table 2).

After fertilization of the egg cells, the first polar body appears after 3 hours. The first cleavage begins after 6.5 hours, and divides the egg into two unequal blastomeres. As a result of the second cleavage after 8.5 hours, four blastomeres are

formed, distributed in a spiral typical of the mollusks. The cleavages that follow divide the micromeres more rapidly than prior to the formation of the spherical blastula, which occurs after 27 hours. Thirty-seven and a half hours after fertilization, a ciliated cover forms in the larva, i.e. the embryo becomes fully formed, and from then on it becomes highly motile. The trochophore forms after 44.5 hours of development. A tuft of long cilia appears in the apical part of the larva, and several long flagella and short cilia form at the animal pole. (9)

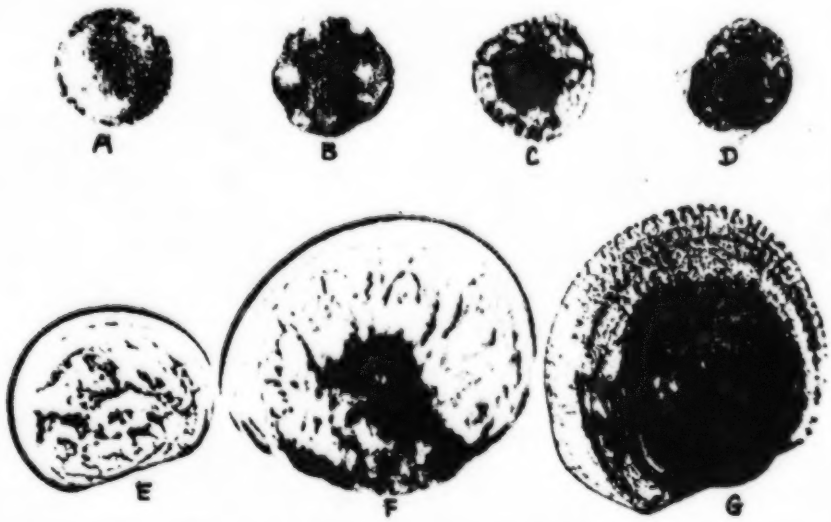

Fig. 2. Early ontogenesis of the Yezo scallop from cells obtained in an artificial environment (A-E - oc. x10, obj. x10; F-G - oc. x10, obj. x20): A - first polar body; B - 16 blastomeres; C - blastula; D - trochophore; E - veliger; F - veliconch; G - spat

An intestine forms in the larva at this time. The trochophore leads an active life. The stage of early veliger begins after 73 hours. A shell measuring up to 120 μm forms after 13 days. The stage of veliconch begins on the 18th day after fertilization. The spat forms after 40 days.

Table 2. Development of the Yezo scallop from genital products obtained under artificial and natural conditions (10°C)

Stage of development	Time at which developmental stages began, hours		Survival of larvae at at different stages of development, %	
	experiment M	control M	experiment M±m	control M±m
Polar body				
primary	3	2.6	95±0.3	93±0.4
secondary	4	4.15	92±0.2	90±0.4
Two blastomeres	6.5	6.5	90±0.2	86±0.2
Four blastomeres	8.5	8.7	88±0.3	83±0.4
Eight blastomeres	12.4	12.3	85±0.3	82±0.5
Sixteen blastomeres	16	15.9	84±0.3	81±0.2
Blastula	27	26.8	81±0.2	80±0.2
Motile embryo	37.5	37.2	79±0.2	78±0.3
Trochophore	44.5	44.2	75±0.3	75±0.1
Early veliger	73	72	69±0.3	70±0.3
Veliger	289	240	60±0.2	54±0.3
Veliconch	436	384	6±0.2	5±0.3
Spat	960	975	2±0.5	1±0.5

(10)

The described early ontogeny of the Yezo scallop is similar to the development of larvae from sex cells obtained from adult scallops during the spawning period.

A comparison of the development of larvae from sex cells obtained under natural and artificial conditions (see table 2) shows that the time at which the developmental stages begin after fertilization and the survival of the larvae at the different stages of development are almost the same in both cases.

Consequently, the genital products obtained in laboratory conditions with the help of heat stimulation are viable, and can be used for artificial reproduction of young Yezo scallops.

Summary

1. Genital products of the Yezo scallop can be obtained much earlier in laboratory conditions with the help of heat stimulation, than in a natural environment.

2. The larval development of the Yezo scallop when the genital products are obtained by heat stimulation does not differ from the development of larvae obtained as a result of natural spawning. This points to the possibility of utilizing tempeature-induced spawning for the reproduction of young scallops.

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